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(21) International Application Number: PCT/US95/02194 (22) International Filing Date: 21 February 1995 (21.02.95) (30) Priority Data: 08/199,771 22 February 1994 (22.02.94) US (71) Applicant: THE SYNTEX-SYNERGEN NEUROSCIENCE JOINT VENTURE [US/US]; 1885 33rd Street, Boulder, CO 80301-2546 (US). (72) Inventors: KNEPP, Victoria; 1229 Pennyroyal Terrace, Sun- nyvale, CA 94087 (US). CALDERWOOD, Thomas; 1355 Cherrywood Square, San Jose, CA 95117 (US). GU, Leo; 12134 Bauchamps Lane, Saratoga, CA 95070 (US). WHAT- LEY, John; Suite 127, 25850 Kay Avenue, Hayward, CA 94545 (US). MUCHNIK, Anna; 1542 Winding Way, Bel- mont, CA 94002 (US). (74) Agents: SWANSON, Barry, J. et al.; Swanson & Bratschun, L.L.C., Suite 200, 8400 E. Prentice Avenue, Englewood, CO 80111 (US).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG). Published <i>With international search report.</i>
(54) Title: PHARMACEUTICAL FORMULATIONS OF CILIARY NEUROTROPHIC FACTOR (57) Abstract Aqueous formulations of ciliary neurotrophic factor (CNTF) suitable for lyophilization and subsequent reconstitution in which rhCNTF is admixed with a bulking agent and a thiol-containing antioxidant are provided.		

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PHARMACEUTICAL FORMULATIONS OF
CILIARY NEUROTROPHIC FACTOR

5 TECHNICAL FIELD OF THE INVENTION

This invention relates to pharmaceutical formulations of ciliary neurotrophic factor suitable for therapeutic treatment of damage to the peripheral nervous system.

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BACKGROUND OF THE INVENTION

The peripheral nervous system consists of those nerve cells that extend axonal processes outside the spinal cord and brain. The principle nerve cell types in the peripheral nervous system are primary motor neurons innervating skeletal muscle and controlling movement, autonomic neurons (both sympathetic and parasympathetic) innervating the cardiovascular system and other internal organs and regulating their function, and sensory neurons innervating sensory receptors throughout the body and conveying sensations including pain and proprioception.

Conditions that compromise the survival and proper function of one or more of these types of peripheral nerve cells cause peripheral nerve damage. Such nerve damage may occur from a wide variety of different causes. Nerve damage may occur through physical injury, which causes the degeneration of the axonal processes and/or nerve cell bodies near the site of injury. Nerve damage may also occur because of temporary or permanent cessation of blood flow to parts of the nervous system, as in stroke. Nerve damage may also occur because of intentional or accidental exposure to neurotoxins, such as the cancer and AIDS chemotherapeutic agents cisplatin and dideoxycytidine (ddC), respectively. Nerve damage may also occur because of chronic metabolic diseases, such as diabetes or renal dysfunction. Nerve damage may also occur because of neurodegenerative diseases such as

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Parkinson's disease, Alzheimer's disease, and Amyotrophic Lateral Sclerosis (ALS), which result from the degeneration of specific neuronal populations.

Neurotrophic factors are naturally occurring proteins that promote the survival and functional activities of nerve cells. Neurotrophic factors have been found in the target cells to which an innervating nerve cell connects. Such target-derived neurotrophic factors regulate the number of contacts formed between innervating nerve cells and the target cell population, and are necessary for the survival and maintenance of these nerve cells.

Neurotrophic factors are also found in cells that are not innervated. An example of such a neurotrophic factor is Ciliary Neurotrophic Factor (CNTF). Human CNTF and the gene encoding human CNTF are described in detail in U. S. patent numbers 4,997,929, and 5,141,856, which are specifically incorporated herein by this reference.

Although the biological role of CNTF has not been conclusively established, CNTF appears to be released upon injury to the nervous system and may limit the extent of injury or neuronal damage. Highly-purified CNTF has been shown to support the survival in cell cultures of chick embryonic parasympathetic, sympathetic, sensory, and motor neurons. There is significant evidence to support that CNTF is a neurotrophic factor for peripheral primary neurons in vivo and in vitro. U. S. patent application serial number 07/735,538 filed July 23, 1991, specifically incorporated herein by reference, shows the surprising effectiveness of systemically administered CNTF to accelerate local recovery at the site of peripheral nerve damage.

The ability of CNTF to protect motor neurons from lesion-induced death may also make it effective in preventing nerve cell degeneration associated with such

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neurodegenerative diseases as Parkinson's disease, Alzheimer's disease, Amyotrophic Lateral Sclerosis (ALS), and Spinal Muscular Atrophy (SMA). U.S. patent application serial number 08/015,218 filed February 8, 1993 and U.S. patent application serial number 08/116,440 filed September 3, 1993, both of which are entitled Methods for Treating Amyotrophic Lateral Sclerosis With CNTF, are specifically incorporated herein by reference. These patents present evidence demonstrating the effectiveness of treating amyotrophic lateral sclerosis in humans with CNTF. In addition, Sendtner et al. (1990) Nature 345:440-441, showed that local application of CNTF prevents lesion-induced death of motor neurons in the rat facial brain stem nucleus. Oppenheim et al. (1991) Science 251:1616-1618, showed that CNTF promoted the in vivo survival of chick spinal motor neurons.

A major problem with the delivery of a therapeutically effective amount of CNTF to a patient for treatment or prevention of peripheral nerve damage is the instability of CNTF in solution. CNTF in solution rapidly degrades via aggregation, deamidation, or oxidation leading to subsequent precipitation. To the extent that any of the protein is degraded, the effective amount of biologically active CNTF may be diminished. Protein integrity must therefore be maintained during manufacture and storage as well as during administration. There are also problems associated with its long term storage from the time of manufacture to administration.

Lyophilization is one method of enabling the long term storage of biological proteins, impeding degradation. However, the lyophilization process itself often presents difficulties. As the volume of liquid decreases during the freezing process, the concentration of formulation excipients increases dramatically, which may denature the protein, reducing

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effective therapeutic activity upon reconstitution. In addition, formation of ice crystals during the freezing process may cause denaturation and also decrease the effective amount of bioactive CNTF available. The most
5 desired formulation for CNTF must be able to prevent degradation of CNTF during the lyophilization process.

This invention provides formulations of CNTF which are stable to lyophilization and reconstitution, and methods of storing biologically active CNTF.

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SUMMARY OF THE INVENTION

The pharmaceutical formulations of this invention suitable for lyophilization comprise aqueous solutions of:

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- (a) ciliary neurotrophic factor;
- (b) a biologically acceptable bulking agent;
- (c) a thiol-containing antioxidant;
- (d) a non-ionic surfactant;
- (e) a buffer; and
- 20 (f) water.

Further embodiments of this invention are the lyophilized formulations from which the water has been substantially removed. Upon reconstitution with a pharmaceutically acceptable reconstituting vehicle, the
25 lyophilized formulations of this invention are suitable for administration to patients in need of therapy.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

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Reference will now be made in detail to the presently preferred embodiments of the invention, which, together with the following examples, serve to explain the principles of the invention.

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The present invention includes therapeutically useful formulations of CNTF for the prevention and treatment of peripheral nerve damage, including neurodegenerative diseases such as Parkinson's and Alzheimer's diseases, ALS, and SMA.

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The lyophilization formulations of CNTF are suitable for maintaining the stability and bioactivity of CNTF when subjected to lyophilization and subsequent reconstitution. The most preferred embodiments of the present invention are aqueous formulations that are stable prior to lyophilization and that have retained their bioactivity following lyophilization, long term storage, and reconstitution.

As used in the specification, "pharmaceutically effective amount" means an amount of CNTF which is therapeutically effective in various administration regimes in the prevention and treatment of peripheral nerve damage. "Biologically acceptable" applies to materials characterized by the absence of significant adverse biological effects in vivo. "Room temperature" is between about 22°C to about 25°C. "Body temperature" is between about 36°C to about 40°C. "Lyophilizable formulation" refers to an aqueous formulation of CNTF which may be freeze dried to a moisture content of less than about 2% and which retains at least about 70% of the initial CNTF bioactivity upon reconstitution. "Isotonic" refers to a solution having approximately the same osmotic pressure as blood serum, about 300 mOsm per liter.

The preferred form of CNTF is human CNTF (hCNTF). The most preferred form of hCNTF is recombinant hCNTF (rhCNTF). Methods of obtaining CNTF suitable for use in the formulations of this invention are known to those skilled in the art. For example, suitable rhCNTF may be produced by the recombinant DNA procedures described in U.S. patent number 5,141,856, specifically incorporated herein by this reference. The CNTF should be at least 65% pure, and most preferably at least 98% pure. The purity of isolated CNTF for use in the formulations may be determined by SDS-PAGE or other means known to those skilled in the art.

The most preferred embodiments of the CNTF

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formulation of the present invention are specifically formulated to remain stable and bioactive during and after lyophilization, and upon reconstitution of the lyophilized material. The lyophilized formulations of this invention are particularly useful for providing long term storage of CNTF. The lyophilizable formulations of this invention comprise CNTF, a biologically acceptable bulking agent, a thiol-containing antioxidant, a non-ionic surfactant, a buffer, and water.

The bulking agent generally provides mechanical support by allowing the matrix to maintain its conformation during and after the freeze drying process. One or more sugars may be used as the bulking agent. Sugars, as used herein, include but are not limited to, monosaccharides, oligosaccharides and polysaccharides, provided that the sugar remains amorphous during the freezing cycle. Examples of suitable sugars include fructose, glucose, mannose, ribose, xylose, maltose, lactose, sucrose, and dextran. Sugar also includes sugar alcohols, such as sorbitol, inositol, dulcitol, xylitol, and arabitol. Mixtures of sugars may also be used in accordance with this invention. The most preferred bulking agent of the present invention is sucrose.

Generally, the lyophilizable formulations of this invention comprise 1 part CNTF, from about 40 to about 2000 parts bulking agent, from about 1 to about 200 parts thiol-containing antioxidant, from about 1 to about 80 parts non-ionic surfactant, and from about 4 to about 200 parts buffer. Optionally, from about 0.01 to about 20 parts chelating agent may be added.

The preferred lyophilizable formulations comprise about 0.05 to 1.2 mg/ml CNTF, 50 to 150 mg/ml sugar, 1 to 5 mg/ml non-ionic surfactant, 0.5 to 10 mg/ml thiol-containing antioxidant, 0.01 to 1.0 mg/ml chelating agent, 0.5 to 10 mg/ml buffer and water, at a pH of

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about 7.0 to about 8.0. The most preferred lyophilizable formulations of CNTF comprise 0.1 to 0.6 mg/ml CNTF, 80 to 90 mg/ml sucrose, about 2 mg/ml polysorbate-80, about 4 mg/ml L-cysteine, about 0.1
5 mg/ml EDTA, and about 2 mg/ml tromethamine, in water, at a pH of about 7.6. An alternative composition comprises the foregoing ingredients having about 0.5 to about 10 mg/ml citric acid substituted for the tromethamine to buffer the solution at a pH of about
10 4.5 to 5.5.

CNTF is formulated as a liquid with a sugar (e.g. sucrose), non-ionic surfactant (e.g. polysorbate 80), chelating agent (e.g. EDTA), and a thiol-containing antioxidant (e.g. L-cysteine). These additives
15 stabilize CNTF during freeze-thaw processes, agitation, and against oxidation; the sugar stabilizes CNTF during freezing by providing a rigid glass in which the protein can disperse. The nonionic surfactant stabilizes CNTF against aggregation by minimizing the
20 surface activity of the protein. Oxidation of CNTF is retarded by a thiol-containing antioxidant which acts as an oxygen substrate, and by a chelating agent that chelates metal ions that can act as pro-oxidants. CNTF solutions must be stored at or below -60°C with a
25 nitrogen headspace at all times (including during shipment) for maximum stability. Prior to compounding, CNTF must be thawed at 2-8°C under nitrogen with gentle mixing. Polysorbate 80 must have low levels of
peroxide to minimize oxidation of CNTF.

30 The lyophilizable formulations of this invention are lyophilized to a residual moisture content of less than about 2%; however, formulations which retain CNTF biological activity at higher or lower amounts or moisture content are also contemplated.

35 The preferred lyophilized formulation comprises from about 0.05 to about 1.2 parts ciliary neurotrophic factor, from about 50 to about 150 parts sugar, from

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about 0.5 to about 10 parts thiol-containing antioxidant, from about 0.5 to about 10 parts buffer, from about 1 to about 5 parts non-ionic surfactant, and less than about 1 part water.

5 The lyophilized CNTF formulation may be reconstituted with water for injection, optionally containing a salt to maintain isotonicity.

10 The lyophilized CNTF formulations of this invention are also useful as a component of a kit to provide a convenient and economical way of providing stable lyophilized CNTF in a form which may be rapidly and easily reconstituted in an appropriate vehicle for administration to a patient in need of treatment. In addition to the lyophilized CNTF formulation, the kits
15 of this invention also comprise a reconstituting vehicle. The reconstituting vehicle may comprise sterile water and a sufficient amount of salt to make the final reconstituted formula essentially isotonic. The reconstituting vehicle may further comprise
20 additional buffer. The total volume of reconstituting vehicle present in the kit should be sufficient to achieve a final CNTF concentration suitable for administration to an individual in need of treatment, i.e. about 0.05 to 1.2 mg/ml CNTF.

25 Regardless of the manner of administration, the specific dose of CNTF is calculated according to the approximate body weight or surface area of the patient. Further refinement of the calculations necessary to determine the appropriate dosage for treatment
30 involving each of the above mentioned formulations is routinely made by those of ordinary skill in the art and is within the scope of tasks routinely performed by them without undue experimentation, especially in light of the dosage information and assays disclosed herein.
35 These dosages may be ascertained through use of the established assays for determining dosages utilized in conjunction with appropriate dose-response data.

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It should be noted that the CNTF formulations described herein may be used for veterinary as well as human applications and that the term "patient" should not be construed in a limiting manner. In the case of veterinary applications, the dosage ranges should be the same as specified above.

EXAMPLE 1. MATERIALS AND METHODS.

Material. The CNTF used in all of the Examples was rhCNTF prepared as described generally in U.S. patent number 5,141,856.

Identification and Quantification of CNTF Using Reverse Phase HPLC (RP-HPLC). Oxidative losses of CNTF under a variety of conditions were determined by RP-HPLC at room temperature. CNTF was identified and quantified by analyzing 10 μ l samples by reversed phase HPLC using a Vydac C18 (5 μ , 4.6 mm x 25 cm) column or a Bakerbond C18 (5 μ , 4.6 X 250 mm) column with a diode array UV detector at 210 nm. The mobile phase was water (0.1% TFA) as Solvent A, and 70:30 acetonitrile: water (0.1% TFA) as Solvent B at a flow rate of 1.0 ml/min for 50 minutes with a gradient profile (A:B) ranging from 80:20 to 30:70 (10 mins.) to 20:80 (30 mins.) to 80:20 (35 mins.).

Identification of CNTF was established by comparing its retention time in the sample with the respective retention time of freshly prepared calibrated standard CNTF solutions made from CNTF from the same lot. The quantity of CNTF in the samples was calculated by comparison to a standard curve obtained with serial dilutions of known concentrations.

Determination of CNTF Concentrations by Cation Exchange Chromatography (CEX). Deamination losses of CNTF under a variety of conditions were determined by room temperature CEX at 210 nm using a Bio-Gel SP-5-W (Bio-Rad) column with a mobile phase of 0.02 M Na₂HPO₄ (pH 6.8) as Solvent A and 0.02 M Na₂HPO₄ + 0.5M NaCl

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(pH 6.8) as Solvent B at 1.0 ml/min for 35 minutes.

The gradient profile (A:B) ranged from 100:0 to 0:100 (20 mins.) to 100:0 (25 min.).

Determination of CNTF Concentrations by Size

- 5 Exclusion Chromatography (SEC). Losses of CNTF through protein aggregation were determined by room temperature SEC at 210 nm using an ULTRASPEROGEL-SEC (Beckman) with a mobile phase of 0.1M KH_2PO_4 + 0.1M Na_2SO_4 (pH 6.8) at 0.5 ml/min. isocratic elution.

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EXAMPLE 2. PREPARATION OF CNTF FORMULATION.

- Into 70 ml water for injection, 8500 mg of sucrose were dissolved with stirring, then in sequence 200 mg of tromethamine, 10 mg EDTA, 200 mg polysorbate 80, and
15 400 mg cysteine dissolved, and the pH adjusted to 7.6 ± 0.2 with 1N sodium hydroxide solution or 1N hydrochloric acid solution. 30 ml water for injection USP were added, the container closed, purged with nitrogen USP, and mixed slowly for a minimum of 10
20 minutes to ensure a homogenous solution. Slowly, 50 mg of CNTF were added to the diluent, and the solution was filtered through two 0.2 μm Millipore Durapore filters at 2-10°C.

25 EXAMPLE 3. LYOPHILIZATION OF CNTF FORMULATION.

- 1.2 ml aliquots of CNTF formulations of Example 2 comprising 0.5 mg/ml rhCNTF, 85 mg/ml sucrose, 2 mg/ml polysorbate 80, 4 mg/ml L-cysteine, 0.1 mg/ml EDTA, and 2 mg/ml tromethamine, at pH 7.6, were placed in 3 ml
30 Type I glass vials covered with lyophilization stoppers. The formulation containing vials were loaded into a freeze dryer chamber (FTS Systems Inc.), which was equilibrated at 5°C prior to the initiation of freezing.

- 35 The shelf temperature was lowered to -5°C over 30 minutes and held at -5°C for 3 hours. The temperature was then lowered to -45°C over 2 hours, 15 minutes and

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held at -45°C for 3 hours. The temperature was then increased to -10°C over 1 hour and maintained at -10°C for 4 hours. The temperature was then decreased to -45°C over 2 hours and held at 45°C for 4 hours. The chamber was evacuated and the pressure was controlled at 100 mTorr with a nitrogen sweep.

The shelf temperature was maintained at -45°C for another 4 hours, then increased to -25°C over 8 hours, 20 minutes and held at -25°C for 20 hours. The temperature was then increased to +30°C over 9 hours, 10 minutes and held at +30°C for 25 hours.

The chamber pressure was increased to 380 mTorr with nitrogen over 15 minutes and the vial was stoppered. The temperature was then decreased to +5°C over 1 hour, 25 minutes and held there until product removal for a maximum of 24 hours.

The freeze-dried powder was stored at 5°C and 25°C and reconstituted at room temperature after 1 month, 3 months, and 11 months with 1 ml of water for injection, buffered to pH 7.6 ± 0.3. Samples were analyzed for CNTF concentration by RP-HPLC, CEX, and SEC. As shown in Table 1, no loss of protein was observed following lyophilization for any of the samples.

EXAMPLE 4. RECONSTITUTION OF LYOPHILIZED FORMULATIONS.

Samples of lyophilized formulations of Example 3 were removed from storage after two months and reconstituted with water for injection.

CNTF concentration determined upon reconstitution and after 24 hours, was unaffected by storage, as shown in Table 2.

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TABLE 1

	STABILITY OF LYOPHILIZED CNTF FORMULATION			
	% Recovery of CNTF as Measured By*:			
	RP-HPLC	RP-HPLC	RP-HPLC	RP-HPLC
Storage Temp (°C)	initial	1 month	3 months	11 months
5	102 ± 2	103.6 ± 2	102.5 ± 2	115 ± 8
25		103.4 ± 2	101.8 ± 2	
	Concentration of CNTF as Measured By*:			
	CEX	CEX	CEX	CEX
Storage Temp (°C)	Initial	1 month	3 months	11 months
5	99 ± 2	105 ± 1	99.8 ± 1	109 ± 2
25		103.6 ± 1	98.8 ± 1	
	Concentration of CNTF as Measured By*:			
	SEC	SEC	SEC	SEC
Storage Temp (°C)	Initial	1 month	3 months	11 months
5	106 ± 3	110.6 ± 2	111.8 ± 4	108 ± 5
25		108.4 ± 2	118.2 ± 2	
*mean ± % RSD				

TABLE 2
STABILITY OF RECONSTITUTED CNTF FORMULATION

Time (hrs)	Storage Condition	Reconstitution Volume (mL)	%LS RP-HPLC†	%LS CEST†
0	5°C	1	102 ± 2	100 ± 3
24	5°C	1	102 ± 3	100 ± 1
0	R.T.	1	102 ± 1	100 ± 3
24	R.T.	1	101 ± 7	100 ± 5
0	R.T.	3	100 ± 2	99 ± 4
24	R.T.	3	100 ± 1	99 ± 3

† %LS = % Label Strength = $\frac{[\text{CNTF}] \text{ at time } T}{\text{nominal } [\text{CNTF}]}$

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WE CLAIM:

1. An aqueous pharmaceutical formulation of ciliary neurotrophic factor, suitable for lyophilization, comprising:

- 5 (a) ciliary neurotrophic factor;
 (b) a biologically acceptable bulking agent;
 (c) a thiol-containing antioxidant;
 (d) a non-ionic surfactant;
 (e) a buffer; and
10 (f) water.

2. A formulation of claim 1 further comprising a chelating agent.

15 3. A formulation of claim 2 buffered to a pH from about 7.0 to about 8.0.

 4. A formulation of claim 2 buffered to a pH from about 4.5 to about 5.5.

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 5. A formulation of claim 1 wherein the bulking agent is a sugar.

 6. A formulation of claim 1 wherein the
25 antioxidant is selected from the group consisting of L-cysteine and glutathione.

 7. A formulation of claim 5 comprising from about 0.05 to about 1.2 mg/ml ciliary neurotrophic factor,
30 from about 50 to about 150 mg/ml sugar, from about 0.5 to about 10 mg/ml thiol-containing antioxidant, from about 0.5 to about 10 mg/ml buffer, and from about 1 to about 5 mg/ml non-ionic surfactant.

35 8. A formulation of claim 1 lyophilized to reduce the moisture content to less than about 2% by weight.

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9. A lyophilized pharmaceutical composition comprising from about 0.05 to about 1.2 parts ciliary neurotrophic factor, from about 50 to about 150 parts sugar, from about 0.5 to about 10 parts thiol-containing antioxidant, from about 1 to about 5 parts non-ionic surfactant, from about 0.5 to about 10 parts buffer, and less than about 1 part water.

10. A process for preparing a formulation of ciliary neurotrophic factor, said formulation exhibiting storage stability of at least one month, comprising:

- (i) preparing an aqueous formulation of claim 1; and
- (j) lyophilizing the aqueous formulation to a moisture content of less than about 2 weight percent.

11. A pharmaceutical composition of ciliary neurotrophic factor comprising a reconstituted solution of a lyophilized formulation of claim 8.

12. A process for inhibiting the oxidation of pharmaceutical formulations of proteins susceptible to oxidative degradation, which process comprises preparing a pharmaceutical formulation of the protein in the presence of an effective amount of a thiol-containing antioxidant.

13. A process of claim 12 wherein the thiol-containing antioxidant is selected from the group consisting of L-cysteine and glutathione.

14. A process of claim 12 wherein the ratio of antioxidant to protein is from about 1:1 to about 200:1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02194

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/00; C07K 1/00

US CL :514/12, 21; 530/350, 399, 417

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12, 21; 530/350, 399, 417

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,218,094 (DELLA VALLE) 08 June 1993, columns 14-16 and claim 10.	1-11
Y	US, A, 5,011,914 (COLLINS ET AL) 30 April 1991, column 3, lines 1-35.	1-5 and 7
Y, P	Proc. Natl. Acad. Sci. USA, Volume 91, issued August 1994, Mayer et al, "N-Acetyl-L-Cysteine is a Pluripotent Protector Against Cell Death and Enhancer of Trophic Factor-Mediated Cell Survival in vitro", pages 7496-7500, especially page 7496.	12-14

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

27 APRIL 1995

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INTERNATIONAL SEARCH REPORT

International application No.

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B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, CAS ONLINE, DERWENT, MEDLINE

Search terms: Ciliary Neurotrophic Factor or CNTF, Pharmaceut?, Lyophiliz? or Freez Dry?,
Antioxidant#, Cysteine or Gluthathione#, Ionic Surfactan?, Chelating Agent#

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